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New Compounds and use thereof

The present invention relates to new compounds that are analogs of PRIMA-1. More particularly, the present invention relates to new compounds for the treatment of disorders and diseases such as, for example cancer, autoimmune diseases and heart diseases.

Background

The most common target for mutations in tumors is the p53 gene. The fact that around half of all human tumors carry mutations in this gene is solid testimony as to its critical role as tumor suppressor. p53 halts the cell cycle and/or triggers apoptosis in response to various stress stimuli, including DNA damage, hypoxia, and oncogene activation (Ko and Prives, 1996; Sherr, 1998). Upon activation, p53 initiates the p53-dependent biological responses through transcriptional transactivation of specific target genes carrying p53 DNA binding motifs. In addition, the multifaceted p53 protein may promote apoptosis through repression of certain genes lacking p53 binding sites and transcription-independent mechanisms as well (Bennett *et al.*, 1998; Gottlieb and Oren, 1998; Ko and Prives, 1996). Analyses of a large number of mutant p53 genes in human tumors have revealed a strong selection for mutations that inactivate the specific DNA binding function of p53; most mutations in tumors are point mutations clustered in the core domain of p53 (residues 94-292) that harbours the specific DNA binding activity (Béroud and Soussi, 1998).

Both p53-induced cell cycle arrest and apoptosis could be involved in p53-mediated tumor suppression. While p53-induced cell cycle arrest could conceivably be reversed in different ways, p53-induced cell death would have advantage of being irreversible. There is indeed evidence from animal in vivo models (Symonds *et al.*, 1994) and human tumors (Bardeesy *et al.*, 1995) indicating that p53-dependent apoptosis plays a major role in the elimination of emerging tumors, particularly in response to oncogenic signaling. Moreover, the ability of p53 to induce apoptosis often determines the efficacy of cancer therapy (Lowe *et al.*, 1994). Taking into account the fact that more than 50% of human tumors carry p53 mutations, it appears highly desirable to restore the function of wild type p53-mediated growth suppression to tumors. The advantage of this approach is that it will allow selective elimination of tumor cells, carrying mutant p53. Tumor cells are particularly sensitive to p53 reactivation, supposedly for two main reasons. First, tumor cells are sensitized to apoptosis due to oncogene activation (reviewed in(Evan and Littlewood, 1998)). Second, mutant p53 proteins tend to accumulate at high levels in tumor cells. Therefore, restoration of the wild type function to the abundant and presumably "activated" mutant p53 should trigger a massive apoptotic response in already sensitized tumor cells, whereas normal cells that express low or undetectable levels of p53 should not be affected. The feasibility of p53 reactivation as an anticancer strategy is supported by the fact that a wide range of mutant p53 proteins are susceptible to reactivation. A therapeutic strategy based on rescuing p53-induced apoptosis should therefore be both powerful and widely applicable.

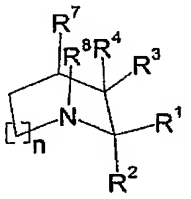
It may be generally shown that malfunctioning of the p53 pathway is generally involved in a number of diesesa, such as those enumerated herein above.

Taken together, these findings suggest that pharmacological restoration of p53 function would result in elimination of tumor cells. Consequently, there is a need within this field to achieve substances and methods for use therein, which enables such a restoration.

The present inventors have found that the compound PRIMA-1 (i.e. 2,2-bis(hydroxymethyl)-1-azabicyclo[2.2.2]octan-3-one) (disclosed in WO0224692), is able to induce apoptosis of cells carrying mutant p53. Later they also found some other analogues to Prima-1 that showed similar results (disclosed in WO03070250). The present inventors now surprisingly have found several new analogues showing even better results, as compared to PRIMA-1 and the earlier analogues, in the treatment of disorders wherein malfunctioning of the p53 pathway may be involved, and this discovery forms the basis of the present invention.

Summary of the invention

According to one aspect, the present invention provides new compounds according to formula (I)



(I)

wherein

n is 0,1 or 2;

R^1 and R^2 are the same or different and are selected from -H, $-CH_2-R^5$, $-CH_2-O-R^5$, $-CH_2-S-R^5$, $-CH_2-NH-R^5$, $-CO-O-R^5$, $-CO-NH-R^5$, $-CH_2-NH-CO-R^5$, $-CH_2-O-CO-R^5$, $-CH_2-NH-CO-NHR^5$, $-CH_2-NH-CO-OR^5$, $-CH_2-NH-CS-NHR^5$ and $-CH_2-O-CO-NHR^5$;

R^3 and R^4 are the same or different and are selected from H, -OH, -SH, $-NH_2$, $-NHR^5$;

or R^3 and R^4 together are =O, =S, or $=NR^5$;

R^5 represents the same or different groups selected from H, substituted or non-substituted C1 to C10 alkyl, C2 to C10 alkenyl, C2 to C10 alkynyl, substituted or non-substituted C3 to C12 cycloalkyl, substituted or non-substituted benzyl groups, substituted or non-substituted aryl or mono-, bi-, tricyclic unsubstituted or substituted heteroaromatic ring(s) with one or more heteroatoms and non-aromatic heterocycles wherein

the substituents of the substituted groups are selected from C1 to C10 alkyl, C2 to C10 alkenyl, C2 to C10 alkynyl, halogen, substituted or non-substituted aryl, substituted or non-substituted hetero-aromatic compounds, non-aromatic heterocycles, C1 to C10 alkyloxy, C1 to C10 alkylamino, C2 to C10 alkenylamino, C2 to C10 alkynylamino, COR^6, $CONR^6$ and $COOR^6$;

R^6 is selected from H, unsubstituted or substituted C1 to C10 alkyl, C2 to C10 alkenyl or alkynyl, benzyl, aryl, unsubstituted or substituted heteroaromatic rings with one or more heteroatoms and non-aromatic heterocycles;

R^7 and R^8 together form a bridging CH_2-CH_2 moiety; or

R^7 and R^8 are both hydrogen;

with the provisos that

- R1 and R2 are not both -H;
- if n=1, R^3 and R^4 together are =O, and R^7 and R^8 together form a bridging CH_2-CH_2 moiety, then R^5 is not H, substituted or non-substituted alkyl or substituted or non-substituted aryl; and

- if n=1, R^3 and R^4 together are =O, R^7 and R^8 together form a bridging CH_2-CH_2 moiety, and R1 or R2 is -H, then R^5 in -CH_2-R^5 is not parasubstituted piperidine, parasubstituted pyrazino, purine, azapurine or benzimidazole;
as well as pharmaceutically acceptable salts or prodrugs of the compounds of formula (I).

According to another aspect, the present invention provides methods of preparing said compounds.

According to a further aspect, the present invention provides the use of the compounds of formula (I) or pharmaceutically acceptable salts or prodrugs thereof for the treatment of diseases associated with mutant p53 or, more generally, a malfunctioning p53 signalling pathway.

According to a still further aspect, the invention provides new pharmaceutical compositions comprising said compounds, or salts or prodrugs thereof.

According to a still further aspect the invention provides a method of medical treatment by use of said pharmaceutical compositions.

According to one aspect, the invention provides the use of the inventive compounds, or salts or prodrugs thereof in the manufacture of a medicament for the treatment or prevention of a disorder selected from hypeproliferative diseases, autoimmune diseases and heart diseases.

Any further aspects are as defined in the claims.

Detailed description of the invention
As used herein the term "lower alkyl" unless otherwise stated, means a unbranched or branched, cyclic, saturated or unsaturated (alkenyl or alkynyl) hydrocarbyl radical which may be substituted or unsubstituted. Where cyclic, the alkyl group is preferably C3 to C12, more preferably C5 to C10, most preferably C5-C7. Where acyclic, the alkyl group is preferably C1 to C10, more preferably C1 to C6, more preferably methyl, ethyl, propyl (n-propyl, isopropyl), butyl (branched or unbranched) or pentyl, most preferably methyl.

As used herein, the term "aryl" means an aromatic group, such as phenyl or naphthyl, or a mono-, bi-, or tricyclic heteroaromatic group containing one or ore heteroatom(s) preferably selected from N, O and S, such as pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, tetrazolyl, isoxazolyl, isothiazolyl, imidazolyl, pyrimidinyl, indolyl, pyrazinyl, indazolyl, pyrimidinyl, thiophenetyl, pyranyl, carbazolyl, acridinyl, quinolinyl, benzoimidazolyl, benzthiazolyl, purinyl, cinnolinyl, pterdinyl.

As used herein, the term "functional groups" means in the case of unprotected: hydroxy-, thiolo-, aminofunction, carboxylic acid and in the case of protected: lower alkoxy, N-, O-, S-acetyl, carboxylic acid ester.

As used herein, the term "heteroaryl" means an aromatic group containing one or more heteroatom(s) preferably selected from N, O and S, such as pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, imidazolyl, pyrimidinyl, indolyl, pyrazinyl or indazolyl.

As used herein, the term "non-aromatic heterocycle" means a non-aromatic cyclic group containing one or more heteroatom(s) preferably selected from N, O and S, such as a cyclic amino group such as pyrrolidinyl, piperidyl, piperazinyl, morpholinyl or a cyclic ether such as tetrahydrofuranyl, monosaccharide.

As used herein the term "halogen" means a fluorine, chlorine, bromine or iodine.

As used herein, and unless specified otherwise, the term "substituted" means that the concerned groups are substituted with functional group such as hydroxyl, amine, sulfide, silyl, carboxylic acid, halogen, aryl, etc.

The compounds according to formula (I) will be useful for treating or preventing various diseases such as hyperproliferative diseases, e.g. cancer, autoimmune diseases, such as rheumatoid arthritis and Sjogren's syndrome, and heart diseases such as hereditary idiopatic cardiomyopathy. The treatment may be preventive, palliative or curative.

Examples of pharmaceutically acceptable addition salts for use in the pharmaceutical compositions of the present invention include those derived from mineral acids, such as hydrochlorid, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids. The pharmaceutically acceptable excipients described herein, for example, vehicles, adjuvants, carriers or diluents, are well-known to those who are skilled in the art and are readily available to the public. The pharmaceutically acceptable carrier may be one which is chemically inert to the active compounds and which have no detrimental side effects or toxicity under the conditions of use. Pharmaceutical formulations are found e.g. in Remington: The Science and Practice of Pharmacy, 19th ed., Mack Printing Company, Easton, Pennsylvania (1995).

Prodrugs of the compounds of formula (I) may be prepared by modifying functional groups present on the compound in such a way that the modifications are cleaved, in vivo when such prodrug is administered to a mammalian subject. The modifications typically are achieved by synthesizing the parent compound with a prodrug substituent. Prodrugs include compounds of formula (I) wherein a hydroxy, amino, sulfhydryl, carboxy or carbonyl group in a compound of formula (I) is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively.

Examples of prodrugs include, but are not limited to, esters and carbamates of hydroxy functional groups, esters groups of carboxyl functional groups, N-acyl derivatives, N-Mannich bases. General information on prodrugs may be found e.g. in Bundegaard, H. "Design of Prodrugs" pl-92, Elesevier, New York-Oxford (1985).

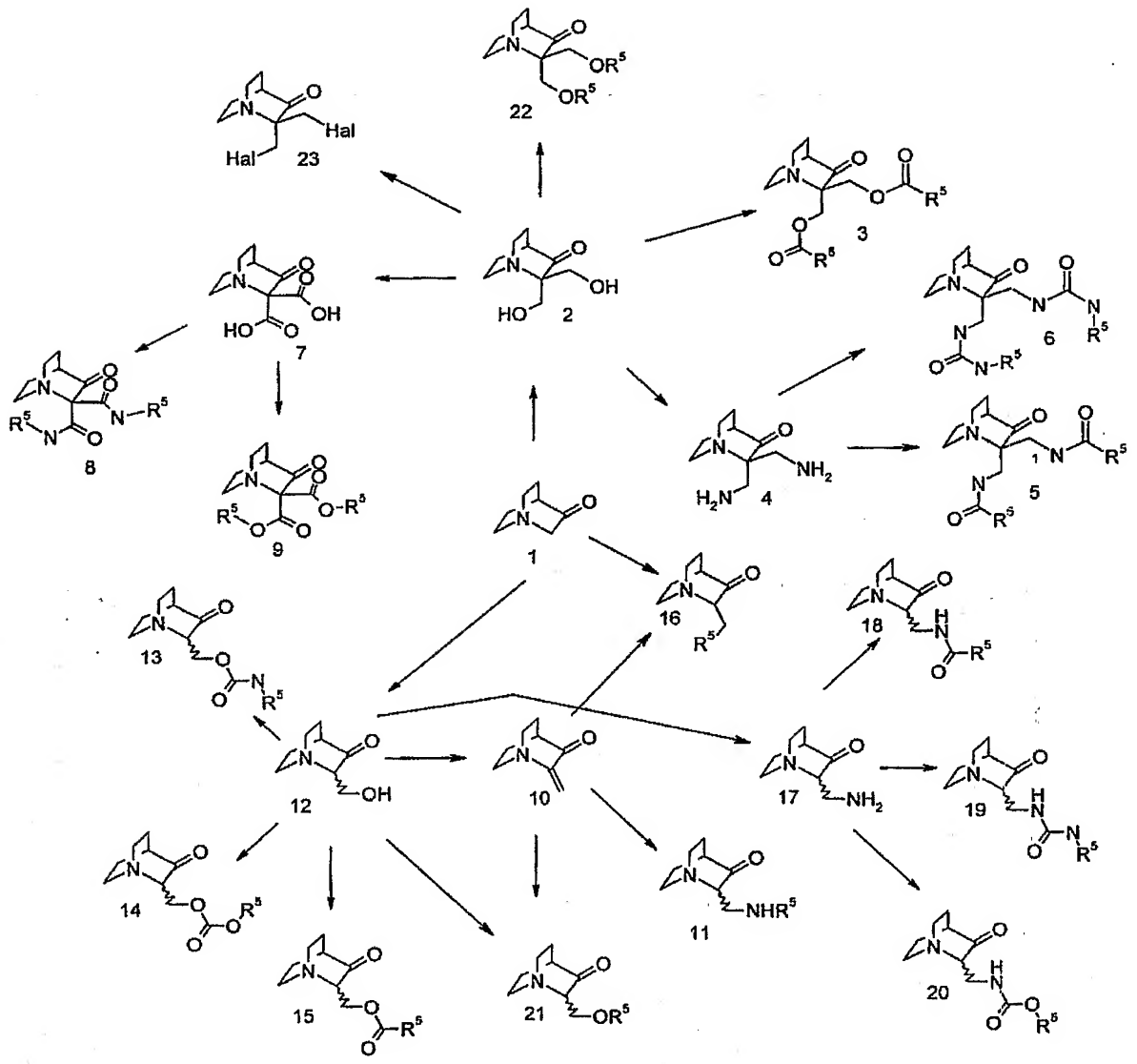
The composition according to the invention may be prepared for any route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, or intraperitoneal. The precise nature of the carrier or other material will depend on the route of administration. For a parenteral administration, a parenterally acceptable aqueous solution is employed, which is pyrogen free and has requisite pH, isotonicity, and stability. Those skilled in the art are well able to prepare suitable solutions and numerous methods are described in the literature. A brief review of methods of drug delivery is also found in e.g. Langer, Science 249:1527-1533 (1990).

The dose administered to an mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the mammal over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors including the potency of the specific compound, the age, condition and body weight of the patient, as well as the stage/severity of the disease. The dose will also be determined by the route (administration form) timing and frequency of administration. In the case of oral administration the dosage can vary from about 0.01 mg to about 1000 mg per day of a compound of formula (I) or the corresponding amount of a pharmaceutically acceptable salt thereof.

The compounds of the present invention may be used or administered in combination with one or more additional drugs useful in the treatment of diseases mediated by mutant p53, or wherein a malfunction of the p53 signalling pathway is involved, such as cytostatic drugs. The components may be in the same formulation or in separate formulations for administration simultaneously or sequentially. The compounds of the present invention may also be used or administered in combination with other treatment such as irradiation for the treatment of cancer.

Examples of cytotstatic compounds for use as indicated herein above are DNA alkylating compounds, topoisomerase I inhibitors, topoisomerase II inhibitors, compounds interfering with RNA and DNA synthesis, compounds polymerising the cytoskeleton, and compounds depolymerising the cytoskeleton. Specific examples are adriamycin, camptothecin and cisplatin.

According to one aspect of the invention, methods of preparing the compounds according to formula (I) are provided. Examples of synthesis of some compounds according to formula (I) are represented in the following reaction scheme 1:



Scheme 1

According to the scheme 1, quinuclidinone hydrochloride (**1**) is used as the starting material for the synthesis of the intermediates (**2**), (**10**) and (**12**). 2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**2**) is formed by treatment of (**1**) with an excess of formaldehyde and potassium carbonate according to methods described by Nielsen et al (J. Org. Chem. 1966, 31, 1053-1057). 2- Hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**12**) is formed by treatment of quinuclidinone hydrochloride with 1 equiv. of formaldehyde and potassium carbonate. 2-Methylene-1-aza-bicyclo[2.2.2]octan-3-one (**10**) is then formed from compound **12** by a dehydration procedure. These methods are also described by Nielsen et al. Compound **10** is also commercially available as its hydrochloride salt.

2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**2**) forms esters by treatment with acid chlorides and a suitable base such as triethylamine, pyridine or DMAP in an organic solvent following standard protocols for acylation. The esterfication may also be performed using carboxylic acids and coupling reagents such as DCC and HOBt.

By using 1 equiv. of the acid chloride a mixture of mono and diacylated products is formed. These products may be separated by preparative HPLC.

Compound **11** and analogs thereof may be formed by reaction between 2-methylene-1-aza-bicyclo[2.2.2]octan-3-one (**10**) and amines in organic solvents and at elevated temperature in the same way as described by Singh et al (J. Med. Chem. 1969, 12, 524-526) or by Elkin en al (US 3726877).

Compounds **4** and **17** may be formed from the corresponding compounds **2** and **12** by conversion of the primary hydroxyl into Boc-protected amines. Boc-deprotection is then performed by standard methods well known for the person skilled in the art, to give compounds **4** and **17** as described by Wood et al (Tetrahedron Lett. 2002, 43, 3887-3890).

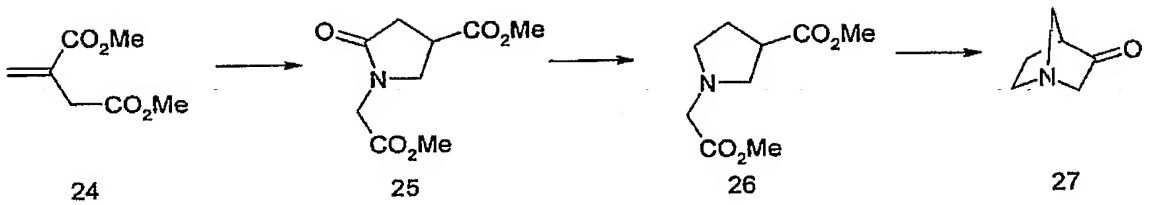
Compound **7** may be formed from 2,2-bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**2**) by oxidation of the primary alcohols to the corresponding carboxylic acids following for example the methods described by Zhao et al (J. Org. Chem. 1999, 64, 2564-2566) using TEMPO as the oxidation reagent or by methods well known for the person skilled in the art.

Compound **22** and analogs thereof may be formed from 2,2-bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**2**) by alkylation, either with alkylhalides as described by Schieweck et al (J. Chem. Soc., Perkin Trans. 1, 2001, 3409-3414) or by the use of orthoester as described by Sampath Kumar et al (Tetrahedron Lett. 1997, 38, 3619-3622).

Compound **16** and analogs thereof may be formed either from compound **1** by reaction between quinuclidinone hydrochloride (**1**) and aldehydes followed by a reduction of the formed double bond according to methods described by for example by Toender et al (Tetrahedron , 2000, 56, 1139-1146) or via compound **10** by a Grignard reaction as described by for example Morgan et al (J. Med. Chem. 1987, 30, 2559-2569) and Sakamuri et al (Tetrahedron Lett. 2000, 41, 9949-9952).

Compound **21** may be formed either by reacting compound **10** with alcohols under basic conditions as described by Nielsen et al (J. Org. Chem. 1966, 31, 1053-1057) or by alkylation of compound **12** with alkyl halides according to Schieweck et al (J. Chem. Soc., Perkin Trans. 1, 2001, 3409-3414).

The synthesis of compounds **5, 6** and **8, 9** from compounds **4** and **7** respectively may be performed by methods well known to the person skilled in the art. That is also true for the formation of compounds **13, 14, 15** and **18, 19, 20** from compounds **12** and **17** respectively.
The corresponding reactions may also be performed on 1-aza-bicyclo[2.2.1]heptane-3-one (**27**) which is based on the scaffold of formula (I) where n= 0:



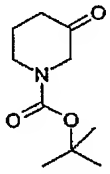
24 25 26 27

Scheme 2

This compound could be synthesized according to methods described by Street et al (J. Med. Chem. 1990, 33, 2690-2697).

The corresponding reactions may also be performed on 1-aza-bicyclo[2.2.3]nonan-3-one which is based on the scaffold of formula (I) where n= 2. The -aza-bicyclo[2.2.3]nonane-3-one could be formed from 1-aza-bicyclo[2.2.2]octan-3-one by ringexpansion as described by Röper et al (Org. Lett. 2000, 1661-1664)

Compounds of formula (I) wherein R^7 and R^8 are both hydrogen may be formed in the same way as for the compounds of formula (I) wherein R^7 and R^8 are an ethylene bridging group starting from Boc-protected 3-keto piperidine (**28**) which may easily be synthesized by method well-known for the person skilled in the art.



(28)

Examples

Synthesis of 2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (2)

The reaction of quinuclidinone hydrochloride (**1**) (commercially available) (16.9 g, 0.1 mol) with formalin (37%w/w, 150 mL, 2.0 mol) in the presence of potassium carbonate (15.9 g, 0.11 mol) consumed the starting material after 1h at 52 °C. Conversion was followed by LC-MS. The water based reaction mixture was extracted with methylene chloride (4x80 mL) and the combined organic phases were dried over $MgSO_4$. The solvent was evaporated off and heptane (500 mL) was added to the residue. After heating for one hour the hot heptane extract was discharged. Benzene (400mL) was added to the residue followed by heating for 8 hours. The resulting mixture was clear filtered from polymer that formed during the heating, and the clear solution was evaporated to dryness. The residue was extracted with boiling heptane (300 mL).

After decanting the heptane, boiling benzene (400 mL) was added to the residue. Clear filtration and cooling (to 6°C) followed by isolation by filtration of the solid precipitate yielded 5.1 g of 2,2-bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (**2**) (mp: 136-138 °C). A second crop was isolated from combined mother liquor and material from heptane extraction after precipitation in benzene yielding 2.9 g of the product. In total 37% yield.

General method for O-acylation of 2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (2) and 2-hydroxymethyl-1-aza-bicyclo[2.2.2]octan-3-one (12)
To a mixture of compound **2** or compound **12** and solid supported dimethylaminopyridine (PS-DMAP) in dry THF 1 equiv. of an acyl chloride is added. The reaction mixture is stirred at room temperature. The reactions are followed by LC-MS. Methylene chloride (DCM) is then added and the mixture is filtered in order to separate the solid supported reagent. The solvent is evaporated and the mono- and di- acylated products are separated and purified by reverse phase preparative HPLC.

General method for N-alkylation of 2-methylene-1-aza-bicyclo[2.2.2]octan-3-one
2-Methylene-1-aza-bicyclo[2.2.2]octan-3-one (**10**) is dissolved un acetone. The N-alkylating agent is added in a slight access and the reaction mixture is heated to reflux for 6 h. The reaction mixture is allowed to reach room temperature and the solvent is evaporated. The product is either purified by recrystallization or by reverse phase preparative HPLC.

General method for O-alkylation of 2-methylene-1-aza-bicyclo[2.2.2]octan-3-one :
O-alkylation may be performed by treating 2-methylene-1-aza-bicyclo[2.2.2]octan-3-one with alcohols and conc. HCl with or without solvent depending on the alcohol. The reaction mixture is stirred at room temperature. The reactions are followed by LC-MS. The products are separated and purified by reverse phase preparative HPLC.

Synthesis of 1-aza-bicyclo[2.2.1]heptane-3-one
Step 1 : Synthesis of Dimethylitaconate
Itaconic acid (125g, 0.946 mol) was taken in 1L of methanol at RT under a nitrogen atmosphere. P-TSA (19g, 0.0998 mol) and conc. H_2SO_4 (2.5 mL) were added and the reaction mixture was then slowly heated to reflux (temperature 68°C) and refluxed for 48h. Completion of reaction was monitored by tlc. The reaction mixture was then concentrated under vacuo and the residue was taken in ethyl acetate (300mL) and basified to pH 8-9 using 10% $NaHCO_3$ solution. The aqueous layer was extracted with ethyl acetate (250mL X 2). The combined ethyl acetate layer was washed with brine , dried and concentrated to get a light yellow oil (119g, 79%).
TLC : methanol:chloroform = 2:8, R_f = 0.8 .

Step 2: Synthesis of 1-methoxy carbonylmethyl-5-oxo-pyrrolidine-3-carboxylic acid methyl ester
Sodium metal (18g, 0.78 mol) was added portionwise to 900mL of methanol under nitrogen atmosphere at RT. The homogeneous mixture was stirred for 0.5h and cooled to 0°C. Glycine methyl ester hydrochloride (100g, 0.8 mol) was added portionwise to the mixture and then dimethyl itaconate (105g, 0.664 mol) was added slowly over 15 minutes at 0°C and heated the reaction mixture and refluxed for ~12h. The precipitate obtained was filtered and the filtrate was concentrated. The residue was taken in 5N HCl (100mL) and extracted with dichloromethane, washed with brine, dried and concentrated to get crude product which was distilled (bp 128-132°C / 0.2mm of Hg) to get a colourless oil (53g, 37%).
TLC: Ethylacetate: Pet-ether 2:8, R_f= 0.3

Step 3: Synthesis of 1-methoxycarbonylmethyl-pyrrolidine-3-carboxylic acid methyl ester
1-methoxycarbonylmethyl-5-oxo-pyrrolidine-3-carboxylic acid methyl ester (52g, 0.24 mol) was taken in 500mL THF under nitrogen and the mixture was cooled at 0°C. BH_3-THF complex (1M in THF, 500mL) was added dropwise over 1h to the reaction mixture at 0°C. Then

the mixture was refluxed for 1h and was stirred for 22h. A saturated solution of K_2CO_3 (33g of K_2CO_3, 0.238 mol) was added and refluxed for another 1h. The solid residue in the reaction mixture was filtered off and the filtrate was concentrated. The concentrated residue was taken onto 5N HCl (120mL) and washed with dichloromethane (2x 300mL). The aqueous layer was basified to pH 8-9 using K_2CO_3 and extracted with dichloromethane, dried and concentrated to get a yellow oil (16g, 32%).
TLC: Dichloromethane, R_f=0.4

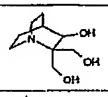
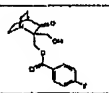
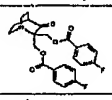
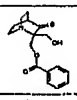
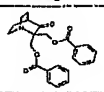
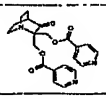
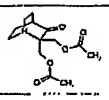
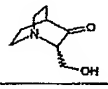
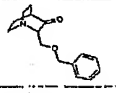
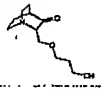
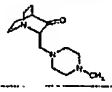
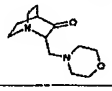
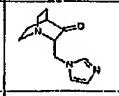
Step 4: Synthesis of 1-aza-bicyclo[2.2.1]heptane-3-one
Potassium-tert-butoxide (23.5g, 0.21 mol) was taken in toluene (600mL) at RT under nitrogen atmosphere and heated the mixture to reflux. The solution of 1-methoxycarbonylmethyl-pyrrolidine-3-carboxylic acid methyl ester (14g, 0.069 mol) in toluene (150mL) was added dropwise over 45 minutes with vigorous stirring and the mixture was allowed to reflux for 6h, cooled to 0°C, conc. HCl (200mL) was added. The organic phase was separated and was washed twice with conc. HCl (200ml x2). The combined aqueous layers was heated at 110°C for 12h, evaporated to half the volume and basified to pH 8-9 using K_2CO_3, extracted with dichloromethane (5x200mL), dried and concentrated and the residue was taken in di-ethylether (300mL) and filtered to remove solids. Filtrate was concentrated to get the product as yellow viscous liquid (2.1g, 27%).
TLC: Ethylacetate:Pet-ether 2:8, R_f= 0.3

2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.1]heptane-3-one
Synthesis of 2,2-Bis-hydroxymethyl-1-aza-bicyclo[2.2.1]heptane-3-one and derivatives from 1-aza-bicyclo[2.2.1]heptane-3-one is performed in the same way as described above for the synthesis of derivatives from 1-aza-bicyclo[2.2.2]octan-3-one.

Biology
Cell lines
The human H1299 lung carcinoma cell lines lacking p53 expression and its transfected clone, H1299 His175 that carry tetracycline-regulated mutant p53 constructs.

WST-1 assay
Cells were plated on 96-well plates at a density of 3000 cells per well per 100ml medium, cultured overnight and treated with 1, 5, 10, 25, and 50μM of compounds. After 96h 10μl of WST-1-cell proliferation reagent were added to each well. Samples were incubated at 37°C for 1-2hs and absorbance of samples was measured at 490nm. Survival of the untreated cells was taken as 100%.

Results from WST-1 assay

ID	Structure	fmla Structure	H1299-His175, Dox-, mtp53 (uM),	H1299, P53 null (uM)	IC50null/IC 50mtp
1		C9H17NO3	>50	>50	inactive
2		C16H18FNO4	12,53	33,66	2,69
3		C23H21F2NO5	14,2	45,58	3,2
4		C16H19NO4	20,01	43,86	2,19
5		C23H23NO5	19,3	42,4	2,2
6		C21H21N3O5	13,52	>50	3,7
7		C13H19NO5	14,85	28,78	1,94
8		C8H11NO	9,83	13,5	1,37
9		C8H13NO2	8,18	13	1,59
10		C15H19NO2	8,49	19,58	2,3
11		C12H21NO2	10,74	19,34	1,9
12		C13H23N3O	8,73	12,34	1,4
13		C12H20N2O2	6,83	18,45	2,7
14		C11H15N3O	6,79	12,98	1,9
15		C10H14N4O	14,58	37,55	2,58

Comparison of the inhibition of proliferation by quinuclidinone analogs in human H1299 lung carcinoma cells lacking p53 expression and in H1299 His175 cells that carry tetracycline-regulated mutant p53 constructs.

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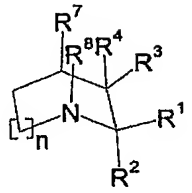
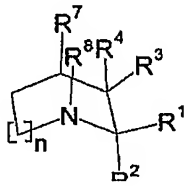
Claims

1. A compound of formula (I)

(I) 

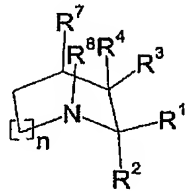
wherein
n is 0,1 or 2;
R^1 and R^2 are the same or different and are selected from -H, $-CH_2-R^5$, $-CH_2-O-R^5$, $-CH_2-S-R^5$, $-CH_2-NH-R^5$, $-CO-O-R^5$, $-CO-NH-R^5$, $-CH_2-NH-CO-R^5$, $-CH_2-O-CO-R^5$, $-CH_2-NH-CO-NHR^5$, $-CH_2-NH-CO-OR^5$, $-CH_2-NH-CS-NHR^5$ and $-CH_2-O-CO-NHR^5$;
R^3 and R^4 are the same or different and are selected from H, -OH, -SH, $-NH_2$, $-NHR^5$;
or R^3 and R^4 together are =O, =S, or $=NR^5$;
R^5 represents the same or different groups selected from H, substituted or non-substituted C1 to C10 alkyl, C2 to C10 alkenyl, C2 to C10 alkynyl, substituted or non-substituted C3 to C12 cycloalkyl, substituted or non-substituted benzyl groups, substituted or non-substituted aryl or mono-, bi-, tricyclic unsubstituted or substituted heteroaromatic ring(s) with one or more heteroatoms and non-aromatic heterocycles wherein
the substituents of the substituted groups are selected from C1 to C10 alkyl, C2 to C10 alkenyl, C2 to C10 alkynyl, halogen, substituted or non-substituted aryl, substituted or non-substituted hetero-aromatic compounds, non-aromatic heterocycles, C1 to C10 alkyloxy, C1 to C10 alkylamino, C2 to C10 alkenylamino, C2 to C10 alkynylamino, COR^6, $CONR^6$ and $COOR^6$;
R^6 is selected from H, unsubstituted or substituted C1 to C10 alkyl, C2 to C10 alkenyl or alkynyl, benzyl, aryl, unsubstituted or substituted heteroaromatic rings with one or more heteroatoms and non-aromatic heterocycles;
R^7 and R^8 together form a bridging CH_2-CH_2 moiety; or
R^7 and R^8 are both hydrogen;
with the provisos that
- R1 and R2 are not both -H;
- if n=1, R^3 and R^4 together are =O, and R^7 and R^8 together form a bridging CH_2-CH_2 moiety, then R^5 is not H, substituted or non-substituted alkyl or substituted or non-substituted aryl; and
- if n=1, R^3 and R^4 together are =O, R^7 and R^8 together form a bridging CH_2-CH_2 moiety, and R1 or R2 is -H, then R^5 in $-CH_2-R^5$ is not parasubstituted piperidine, parasubstituted pyrazino, purine, azapurine or benzimidazole;
or a pharmaceutically acceptable salt or prodrug thereof.

2. A process for the preparation of a compound according to claim 1 by N-alkylating, O-alkylating or S-alkylating a compound of formula (I)

(I) 

wherein n, R^3, R^4, R^7 and R^8 are as defined in claim 1 and R^1 and R^2 together form a methylene group.

3. A process for the preparation of a compound according to claim 1 by acylating or alkylating a compound of formula (I)



(I)

wherein n, R^3, R^4, R^7 and R^8 are as defined in claim 1 and at least one of R^1 and R^2 comprises an amine, hydroxyl or thiol functionality.

4. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt or prodrug thereof, and at least one pharmaceutically acceptable excipient.

5. A compound according to claim 1 for use as a medicament.

6. Use of a compound according to claim 1 for preparing a medicament for the treatment of a disorder selected from hyperproliferative diseases, autoimmune diseases, and heart diseases.

7. The use according to claim 6, wherein the disorder is a cancer.

8. A method of treatment of a disease selected from hyperproliferative diseases, autoimmune diseases, and heart diseases by administration of a therapeutically effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt or prodrug thereof to a patient in the need of such treatment.

Abstract

The invention relates to novel PRIMA-1 analogs, a process for preparing them and a pharmaceutical composition comprising them. The compounds may be used for hyperproliferative diseases, e.g. cancer, autoimmune diseases, and heart diseases.